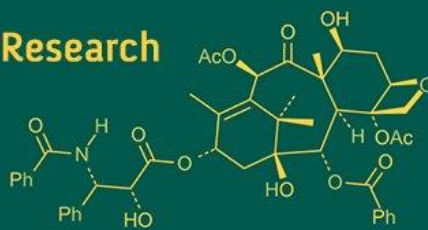


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Variability in cultural and morphological characteristics of *Macrophomina phaseolina* isolates associated with dry root rot of mungbean

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Abstract

Dry root rot of mungbean, caused by *Macrophomina phaseolina*, is influenced by considerable variability in the cultural and morphological characteristics of the pathogen. Thirty isolates associated with dry root rot of mungbean were characterized for their cultural and morphological variability under in vitro conditions. Considerable variation was observed among the isolates with respect to colony growth, texture, colour, margin, sclerotial production, size and shape. After 10 days of incubation on Potato Dextrose Agar, colony diameter ranged from 85.55 to 90.00 mm, with the maximum growth recorded in Mp 23 (Manpur) and Mp 9 (Khadpipli), while Mp 8 (Mahobatpur) exhibited the minimum growth. The isolates displayed diverse colony textures, including fluffy, woolly, velvety, cottony and compact growth, along with considerable variation in colony colour and margin characteristics. Sclerotial production varied from poor to excellent, while sclerotial size ranged from 49.58–75.69 to 88.45–115.67 µm, with Mp 23 producing the largest sclerotia. Variation in sclerotial shape was also evident, ranging from circular and oval to irregular forms. Overall, the findings revealed substantial cultural and morphological variability among *M. phaseolina* isolates associated with dry root rot of mungbean.

Keywords: *Macrophomina phaseolina*, cultural variability, morphological variability, sclerotia, mungbean

1. Introduction

Pulses are recognized as nutritionally valuable food legumes and form an integral part of diets in many developing countries, particularly in India. Their consumption alongside cereal-based foods provides a balanced source of dietary protein and contributes to improved nutritional quality (Ali and Sen Gupta, 2012; Calles *et al.*, 2019; Ramya *et al.*, 2026)^[1, 3, 7]. Beyond their role in human nutrition, pulse crops have considerable agronomic significance owing to their ability to fix atmospheric nitrogen through symbiotic associations, enhance soil fertility and perform relatively well under low-input production conditions. Their importance in sustainable food production and nutritional security was globally acknowledged through the declaration of 2016 as the “International Year of Pulses” by the United Nations General Assembly (Khan, 2016)^[6].

Among the food legumes cultivated in Asia, mungbean [*Vigna radiata* (L.) Wilczek] occupies an important position because of its short growth cycle, nutritional value and relatively low requirement for external inputs. The crop, commonly referred to as green gram, is widely cultivated throughout India and several countries of Southeast and East Asia (Lambrides and Godwin, 2017)^[10]. Mungbean belongs to the family Leguminosae and is considered to have originated in the Indian subcontinent, where its domestication dates back several centuries (Vavilov, 1926)^[20]. It is predominantly suited to tropical and subtropical climates and can be grown across diverse geographical regions where temperatures remain above approximately 15 °C (Lawn, 1983; Imrie *et al.*, 1981)^[11, 5]. Its early maturity and flexibility in cropping sequences further enhance its importance in intensive agricultural systems.

However, the productivity potential of mungbean is frequently affected by a range of environmental and biological stresses. Among these, diseases represent a major limitation to crop establishment, growth and yield. Several fungal, bacterial and viral diseases have been documented on mungbean, including yellow mosaic disease, *Cercospora* leaf spot, powdery

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mildew, anthracnose, web blight, bacterial blight and dry root rot (Khattak *et al.*, 2000; Lal, 1987; Pandey *et al.*, 2018; Roy *et al.*, 2023) [8, 9, 14, 18]. The occurrence and intensity of these diseases are influenced by the interaction among host genotype, pathogen population and prevailing environmental conditions, which can result in substantial variation in disease expression across locations and growing seasons.

Dry root rot, caused by the soil- and seed-borne fungus *Macrophomina phaseolina* (Tassi) Goid, has emerged as an important disease of mungbean, particularly in regions characterized by high temperature and inadequate soil moisture. The pathogen possesses a broad host range and has been reported from several important mungbean-producing areas, where infection may result in considerable yield losses (Allen *et al.*, 2017; Pandey *et al.*, 2020) [2, 13]. A major factor contributing to the persistence of *M. phaseolina* is its ability to produce microsclerotia, which remain viable in soil and infected crop residues for prolonged periods and serve as important sources of primary inoculum (Pandey *et al.*, 2021) [15].

Infected plants commonly exhibit deterioration of the root system, followed by darkening and rotting of roots, yellowing, wilting and eventual death under severe disease pressure (Khan *et al.*, 2017) [7]. Disease development is closely associated with environmental conditions, particularly elevated temperature and reduced soil moisture, which favor infection and colonization by the pathogen (Saleh *et al.*, 2010) [19]. In addition, the movement of infested soil, infected residues and contaminated seed can facilitate the dissemination and establishment of the pathogen within and between production areas. The prolonged survival of microsclerotia in soil further complicates disease management and contributes to the recurring nature of the disease.

An important characteristic of *M. phaseolina* is its capacity to exhibit considerable variation in biological and phenotypic traits among isolates. Such variability may be reflected in colony characteristics, growth rate, pigmentation, microsclerotial production and other morphological attributes. Understanding this variability is particularly important because differences among pathogen populations may influence disease development, host-pathogen interactions and the effectiveness of disease management practices. Characterization of the pathogen population therefore provides useful baseline information for subsequent studies on pathogenicity, epidemiology and management.

Management of mungbean dry root rot remains difficult because of the soil-borne nature of the pathogen, long-term survival of microsclerotia and favorable environmental conditions that promote disease development. Various approaches, including chemical fungicides, biological control agents and other cultural or integrated practices, have been explored to reduce disease incidence. Nevertheless, sustainable disease suppression requires a comprehensive understanding of the pathogen and its biological variability before appropriate management strategies can be developed and optimized.

Therefore, the present investigation was undertaken to characterize *M. phaseolina* isolates associated with dry root rot of mungbean, with particular emphasis on their cultural and morphological variability and pathogenic potential. The study is intended to provide a better understanding of the

variability present within the pathogen population and generate information useful for the development of effective and sustainable management strategies for mungbean dry root rot.

2. Materials and Methods

2.1 Cultural Studies

The present study aimed to evaluate the cultural variability of thirty isolates of *M. phaseolina* (Table 1). The isolates of *M. phaseolina* were purified through the hyphal tip technique on PDA slants and stored in a refrigerator (4 °C). It was then grown separately on PDA in Petri plates for seven days to provide inoculum for the study. Cultural studies for the isolate were conducted on PDA in 90 mm Petri plates. The Petri plates were inoculated centrally with 5 mm culture discs taken from the periphery of a seven-day-old culture and incubated at 28 ± 2 °C. Observations on cultural characters, viz., colony colour, type, growth, size and shape, were recorded one week after inoculation. Morphological characters of the pathogen were studied by examining the culture under a compound microscope.

2.2 Morphology of *M. phaseolina*

Slides of the fungus were prepared by growing hyphae or the respective samples. The fungal material was mounted on a clean glass slide with a drop of lactophenol cotton blue and covered with a coverslip. The morphological characters of the fungus were studied with the help of a compound microscope (40X). After calibrating the microscope, measurements of hyphae, sclerotia, were taken using ocular and stage micrometers.

Table 1: List of *M. phaseolina* isolates collected from different talukas and villages

Sr. No.	Isolate	Taluka	Villages/City
1	MP-1	Junagadh	Makhiyala
2	MP -2		Makhiyala
3	MP -3		Navagam
4	MP -4		Vadal
5	MP -5		Sukhpur
6	MP -6		Chokli
7	MP -7	Vanthali	Naredi
8	MP -8		Mahobatpur
9	MP -9		Luvarsar
10	MP -10		Selra
11	MP -11		Sonaradi
12	MP -12		Sonaradi
13	MP-13	Mendarda	Khadpipli
14	MP -14		Alidhra
15	MP -15		Alidhra
16	MP -16		Mendarda
17	MP -17		Manpur
18	MP -18		Kariya
19	MP -19	Bhesan	Hadmatiya Vishal
20	MP -20		Hadmatiya Vishal
21	MP -21		Kharchiya
22	MP -22		Mendapara
23	MP -23		Ranpur
24	MP -24		Bantva
25	MP -25	Manavadar	Galvav
26	MP -26		Manavadar
27	MP -27		Manavadar
28	MP -28		Marmath
29	MP -29		Nakra
30	MP -30		Khadpipli

3. Results and Discussion

3.1 Cultural and Morphological Variability of *Macrophomina phaseolina* Isolates

Thirty isolates of *Macrophomina phaseolina* obtained from different locations of Junagadh district were evaluated for their cultural and morphological characteristics under *in vitro* conditions. Considerable variability was observed among the isolates with respect to radial growth, colony texture, colony colour, colony margin, sclerotial production, sclerotial size and sclerotial shape (Table 2).

3.1.1 Radial growth

Marked variation in radial growth was observed among the thirty isolates after 10 days of incubation. Colony diameter ranged from 85.55 to 90.00 mm. The maximum colony diameter of 90.00 mm was recorded in Mp 9 (Khadpipli) and Mp 23 (Manpur), whereas the minimum colony diameter of 85.55 mm was recorded in Mp 8 (Mahobatpur). The variation in radial growth indicated differences in the growth potential of the *M. phaseolina* isolates under the given *in vitro* conditions.

3.1.2 Colony texture and growth pattern

Considerable variation was observed in colony texture and growth pattern among the isolates. Aerial, fluffy and abundant growth was recorded in Mp 1 (Makhiyala), Mp 8 (Mahobatpur), Mp 14 (Alidhra) and Mp 25 (Nakra). Aerial with partially submerged growth was observed in Mp 2 (Naredi), Mp 4 (Kariya), Mp 12 (Vadal) and Mp 26 (Makhiyala). Compact and partially submerged growth was recorded in Mp 5 (Bantva) and Mp 6 (Kevadra). Aerial and woolly growth was observed in Mp 9 (Khadpipli), Mp 17 (Sukhpur), Mp 23 (Manpur) and Mp 28 (Alidhra). Aerial velvety to compact and sparse growth was observed in Mp 10 (Hadmatiya Vishal) and Mp 24 (Ranpur), whereas cottony growth was recorded in Mp 13 (Luvarsar), Mp 21 (Chokli) and Mp 27 (Mahobatpur).

3.1.3 Colony colour

Substantial variation in colony colour was observed among the isolates. Brownish-grey colonies were produced by Mp 1 (Makhiyala), Mp 4 (Kariya) and Mp 22 (Sonaradi). Greyish-white colonies were observed in Mp 2 (Naredi), Mp 8 (Mahobatpur), Mp 11 (Galvav), Mp 16 (Manavadar), Mp 18 (Selra) and Mp 26 (Makhiyala). Olive-grey colonies were produced by Mp 3 (Mithapur), Mp 12 (Vadal), Mp 29 (Mendapara) and Mp 30 (Nakra). Pale-grey colonies were observed in Mp 6 (Kevadra), Mp 15 (Kharchiya), Mp 17 (Sukhpur) and Mp 23 (Manpur). Grey colonies were recorded in Mp 9 (Khadpipli), Mp 13 (Luvarsar) and Mp 27 (Mahobatpur). Blackish-grey colonies were produced by Mp 7 (Navagam), Mp 10 (Hadmatiya Vishal), Mp 19 (Mendarda) and Mp 28 (Alidhra). Dark-grey colonies were observed in Mp 14 (Alidhra) and Mp 20 (Marmath), whereas whitish-grey colonies were recorded only in Mp 21 (Chokli).

3.1.4 Colony margin

Variation in colony margin was also evident among the isolates. The majority of isolates exhibited regular colony margins. Irregular margins were observed in Mp 4 (Kariya), Mp 8 (Mahobatpur), Mp 15 (Kharchiya), Mp 19 (Mendarda) and Mp 26 (Makhiyala). Rough margins were recorded in Mp 16 (Manavadar), Mp 24 (Ranpur) and Mp 30 (Nakra), whereas smooth margins were observed in Mp 13 (Luvarsar), Mp 18 (Selra), Mp 21 (Chokli), Mp 25 (Nakra) and Mp 29 (Mendapara).

3.1.5 Sclerotial production

Considerable variation was observed in the degree of sclerotial production among the *M. phaseolina* isolates. Excellent sclerotial production (++++) was recorded in Mp 2 (Naredi), Mp 5 (Bantva), Mp 6 (Kevadra), Mp 9 (Khadpipli), Mp 12 (Vadal), Mp 14 (Alidhra), Mp 17 (Sukhpur), Mp 19 (Mendarda), Mp 23 (Manpur) and Mp 28 (Alidhra). Good sclerotial production (+++) was observed in Mp 1, Mp 3, Mp 10, Mp 11, Mp 16, Mp 20, Mp 24, Mp 25, Mp 27 and Mp 30. Moderate sclerotial production (++) was recorded in Mp 4, Mp 7, Mp 13, Mp 15, Mp 18, Mp 21, Mp 26 and Mp 29. Poor sclerotial production (+) was observed in Mp 8 (Mahobatpur) and Mp 22 (Sonaradi).

3.1.6 Sclerotial size

The isolates exhibited considerable variation in the size of sclerotia. The overall sclerotial size ranged from 49.58–75.69 to 88.45–115.67 μm . The largest sclerotia were produced by Mp 23 (Manpur), measuring 88.45–115.67 μm , followed by Mp 9 (Khadpipli) with 86.32–110.57 μm and Mp 28 (Alidhra) with 85.12–108.56 μm . The smallest sclerotia were recorded in Mp 5 (Bantva), with dimensions of 49.58–75.69 μm . These observations indicate appreciable variation in sclerotial dimensions among the isolates.

3.1.7 Sclerotial shape

Variation was also observed in the shape of sclerotia produced by different isolates. Circular to oval-shaped sclerotia were predominant and were recorded in Mp 3, Mp 7, Mp 10, Mp 11, Mp 15, Mp 20, Mp 24 and Mp 27. Spherical to oval-shaped sclerotia were observed in Mp 1, Mp 9 and Mp 17, whereas spherical to oblong-shaped sclerotia were recorded in Mp 6 (Kevadra). Circular to irregular-shaped sclerotia were observed in Mp 8 (Mahobatpur) and Mp 22 (Sonaradi). Irregular-shaped sclerotia were produced by Mp 2, Mp 4, Mp 5, Mp 12, Mp 16, Mp 19, Mp 26 and Mp 30.

3.1.8 Overall variability among isolates

Overall, the thirty *M. phaseolina* isolates exhibited considerable phenotypic variability in both cultural and morphological characteristics. Variations were observed in radial growth, colony texture, colour and margin, as well as in the production, size and shape of sclerotia. Such variability among isolates collected from different locations of Junagadh district indicates the occurrence of considerable intraspecific variation in *M. phaseolina* and provides a useful basis for further studies on pathogenic variability and disease management.

Table 2: Cultural and morphological variability among the isolates of *M. phaseolina* under *in vitro* condition

Sr. No.	Isolate	Colony diameter (mm)	Colony appearance			Sclerotia		
			Texture	Colour	Margin	Production*	Size (µm)	Shape
1	Mp 1 Makhiyala	89.32	Aerial, fluffy and abundant	Brownish Grey	Regular and smooth	+++	62.21–75.41	Spherical to oval
2	Mp 2 Naredi	87.61	Aerial and partial submerged	Greyish white	Regular	++++	53.41–85.03	Irregular
3	Mp 3 Mithapur	87.00	Aerial and fluffy	Olive grey	Regular	+++	70.56–85.25	Circular to oval
4	Mp 4 Kariya	86.70	Aerial and partial submerged	Brownish grey	Irregular	++	58.96–94.13	Irregular
5	Mp 5 Bantva	89.54	Aerial, compact and partial submerged	Dark grey	Irregular and flat	++++	49.58–75.69	Irregular
6	Mp 6 Kevadra	89.00	Aerial, compact, partial submerged and sparse	Pale grey	Irregular and rough	++++	80.37–94.41	Spherical to oblong
7	Mp 7 Navagam	88.22	Aerial, fluffy and sparse	Blackish grey	Regular and smooth	++	68.28–71.53	Circular to oval
8	Mp 8 Mahobatpur	85.55	Aerial, fluffy and abundant	Greyish white	Irregular	+	54.61–97.52	Circular to irregular
9	Mp 9 Khadpipli	90.00	Aerial, woolly and profuse	Grey	Regular	++++	86.32–110.57	Spherical to oval
10	Mp 10 Hadmatiya Vishal	87.00	Aerial, velvety and sparse	Blackish grey	Regular	+++	65.32–79.47	Circular to oval
11	Mp 11 Galvav	88.20	Aerial, fluffy and dense	Greyish white	Regular	+++	60.12–82.45	Circular to oval
12	Mp 12 Vadai	86.75	Aerial and partial submerged	Olive grey	Regular	++++	72.34–95.12	Irregular
13	Mp 13 Luvarsar	87.40	Aerial, cottony	Grey	Smooth	++	55.21–78.34	Circular
14	Mp 14 Alidhra	89.10	Aerial, fluffy and abundant	Dark grey	Regular	++++	80.45–102.11	Spherical
15	Mp 15 Kharchiya	85.95	Aerial and sparse	Pale grey	Irregular	++	62.13–74.56	Circular to oval
16	Mp 16 Manavadar	88.60	Aerial, compact and dense	Greyish grey	Rough	+++	68.22–89.45	Irregular
17	Mp 17 Sukhpur	87.80	Aerial, woolly	Pale grey	Regular	++++	84.11–110.22	Spherical to oval
18	Mp 18 Selra	86.40	Aerial, fluffy	Greyish white	Smooth	++	58.34–79.55	Circular
19	Mp 19 Mendarda	89.25	Aerial, compact	Blackish grey	Irregular	++++	75.66–98.34	Irregular
20	Mp 20 Marmath	88.10	Aerial and submerged	Dark grey	Regular	+++	64.12–85.67	Circular to oval
21	Mp 21 Chokli	87.55	Aerial, cottony and sparse	Whitish grey	Smooth	++	59.78–73.22	Circular
22	Mp 22 Sonaradi	85.80	Aerial, fluffy	Brownish grey	Regular	+	52.11–69.34	Circular to irregular
23	Mp 23 Manpur	90.00	Aerial, woolly and dense	Pale grey	Regular	++++	88.45–115.67	Spherical
24	Mp 24 Ranpur	87.00	Aerial, compact and sparse	Greyish white	Rough	+++	66.34–83.12	Circular to oval
25	Mp 25 Nakra	88.45	Aerial, fluffy and abundant	Pale grey	Smooth	+++	61.78–79.90	Circular
26	Mp 26 Makhiyala	86.90	Aerial and partial submerged	Greyish white	Irregular	++	57.23–76.44	Irregular
27	Mp 27 Mahobatpur	87.75	Aerial, cottony	Grey	Regular	+++	63.45–82.78	Circular to oval
28	Mp 28 Alidhra	89.35	Aerial, woolly	Dark grey	Regular	++++	85.12–108.56	Spherical
29	Mp 29 Mendapara	88.00	Aerial, fluffy	Blackish grey	Smooth	++	60.22–74.33	Circular
30	Mp 30 Nakra	86.60	Aerial, compact	Olive grey	Rough	+++	67.89–88.23	Irregular

Discussion

The present investigation revealed considerable variation in the cultural and morphological characteristics of the *Macrophomina phaseolina* isolates grown on PDA medium. The isolates differed in several phenotypic attributes,

including colony colour, colony texture, growth pattern, degree of pigmentation, sclerotial production and sclerotial size. Such variation indicates the presence of substantial phenotypic diversity within the *M. phaseolina* population associated with mungbean dry root rot. Differences in

colony morphology and growth behaviour among isolates may reflect inherent variability in their physiological and adaptive characteristics.

The findings of the present study are in close agreement with the observations of Aghakhani and Dubey (2009), Manjunatha and Naik (2011), and Patra and Biswas (2017), who documented considerable cultural and morphological variability among *M. phaseolina* isolates maintained on PDA medium. These researchers reported differences in colony colour, texture, growth pattern and sclerotial characteristics, supporting the occurrence of considerable phenotypic variation within the pathogen. The variability observed in the present study, particularly with respect to colony appearance and sclerotial characteristics, further corroborates these earlier observations.

Variation in sclerotial production and size is of particular importance because microsclerotia constitute the principal survival structures of *M. phaseolina* and enable the pathogen to persist in soil and infected crop residues. Differences in the abundance and size of sclerotia among isolates may therefore reflect variation in their survival and adaptive capacity under different environmental conditions. Likewise, differences in radial growth and colony morphology may be associated with variations in the physiological behaviour of individual isolates.

Overall, the consistency between the present findings and earlier reports suggests that cultural and morphological variability is a common characteristic of *M. phaseolina* populations. Such variability may have important implications for pathogen survival, disease development and host–pathogen interactions. Characterization of this variation is therefore essential for understanding the biology and epidemiology of *M. phaseolina* and may provide useful baseline information for subsequent investigations on pathogenic variability and the development of effective disease management strategies.

Conclusion

The present investigation demonstrated considerable cultural and morphological variability among the thirty isolates of *Macrophomina phaseolina* associated with dry root rot of mungbean under *in vitro* conditions. The isolates differed considerably in colony growth, texture, colour, margin characteristics, sclerotial production, size and shape, indicating substantial phenotypic diversity within the pathogen population.

Colony diameter after 10 days of incubation ranged from 85.55 to 90.00 mm, with Mp 23 (Manpur) and Mp 9 (Khadpipli) recording the maximum growth, whereas Mp 8 (Mahobatpur) exhibited the minimum growth. The isolates showed diverse colony textures, including fluffy, woolly, cottony, velvety and compact types, along with variation in colony colour and margin characteristics. Sclerotial production varied from poor to excellent among the isolates, while sclerotial size and shape also exhibited marked differences. The largest sclerotia were recorded in Mp 23 (Manpur), whereas the smallest were observed in Mp 5 (Bantva).

Overall, the study confirmed pronounced cultural and morphological variability among *M. phaseolina* isolates from mungbean. Such variability may contribute to differences in the survival, adaptation and disease-causing ability of the pathogen. The characterization of these isolates provides valuable baseline information for further

studies on pathogenic variability, genetic diversity, host–pathogen interactions and the development of effective management strategies for dry root rot of mungbean.

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